

CLAIMS:

- 5 1. An assay for trypsin inhibitors in urine which comprises contacting a urine test sample with a buffered assay medium comprising trypsin, a substrate for trypsin which will produce a detectable response when cleaved by trypsin and a polycarboxylic chelating agent in sufficient quantity to inhibit interference with the assay from calcium present in the urine and
- 10 correlating the concentration of trypsin inhibitor with the detectable response from the cleaving of the substrate.
- 15 2. The assay of Claim 1 wherein the assay reagents are in solution.
- 20 3. The assay of Claim 2 wherein the solvent used to form the solution is an aqueous or polar aprotic solvent.
- 25 4. The assay of Claim 3 wherein the solvent is water, ethanol, methanol, isopropanol, acetonitrile, dimethyl sulfoxide, acetone, dimethylformamide or methylethylketone.
5. The assay of Claim 1 wherein the assay reagents are in <sup>a</sup>the dry phase.

6. The assay of Claim 5 wherein the assay reagents are impregnated into a dry test device of a material through which the urine test sample can flow by dipping the dry test device into the buffered assay medium with subsequent drying of the solvent.

7. The assay of Claim 1 wherein the chelating agent is ethylene glycol bis ( $\beta$ -aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA); ethylenediaminetetraacetic acid (EDTA); iminodiacetic acid (IDA); nitrilotriacetic acid (NTA); diethylenetriaminopentaacetic acid (DTPA); triethylenetriamine-hexa-acetic acid (TTHA); 2,3-propylenediamino-tetra-acetic acid (UEDTA) and 1,2-diaminocyclohexanetetra-acetic acid.

8. The assay of Claim 1 wherein the trypsin is present in an amount of from 10 to 750 IU/mL, the chelating agent is present in an amount of from 0.2 to 50 mM, the trypsin substrate is present in a concentration of from 0.2 to 50 mM and the pH is buffered at a level of from 6.0 to 8.0.

9. The assay of Claim 8 wherein the trypsin concentration is from 100 to 500 IU/mL, the chelating agent is present in a concentration of from 10 to 25 mM, and the pH is at a level of from 7.0 to 8.0.

10. The method of Claim 1 wherein the substrate for trypsin is selected from the group consisting of arginine or lysine derivatives of 7-amino-4-methylcourmarin, 2-aminonaphthalene, 4-methoxy-2-amino-  
5 naphthalene, 3-carboxy-4-hydroxy-aniline, 2-chloro-4-nitro-aniline, 3-aminoindole, 2-aminoacridone, 2-aminobenzothiazole, 2-aminopyrimidine, Rhodamine 110 and 6-aminoquinoline.
- 10 11. A method for preparing a test device for the determination of trypsin inhibitor in urine which comprises contacting a pad of absorbant material with an aqueous solution of trypsin and a poly carboxylic chelating agent followed by drying the strip and contact-  
15 ing it with a solvent solution of a substrate for trypsin with subsequent drying.
- 20 12. The method of Claim 11 wherein the solvent solution of Claim 11 contains a non-ionic polyoxyalkyl surfactant.
13. The method of Claim 12 wherein the surfactant contains ethylene glycol units.
- 25 14. The method of Claim 11 wherein the trypsin substrate is 3-(N $\alpha$ -tosyl-N $\epsilon$ -nitro-L-arginyloxy)-5-phenylpyrrole.